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Anti-HIV Vaccines
Current Status and Future Developments

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Despite the most intensive scientific and social efforts to stem the AIDS epidemic, the spread of HIV-1 continues with apparent impunity. The World Health Organization (WHO) estimates that by the end of this decade the cumulative total of HIV-infected people in the world will reach 30 to 40 million. The vast majority of these infections occur or will occur in relatively underdeveloped regions, countries woefully unprepared for such an impending medical disaster. Even in the US, 1% of the male population presently carries the HIV, and as it seems that most, if not all, HIV-infected patients eventually succumb to the prolonged and ultimately lethal series of infections characteristic of AIDS, the burden on healthcare facilities even there will be immense.

The therapeutic value of many of the anti-HIV drugs is at best questionable, and with the standard treatment zidovudine being recently apparently discredited for use in healthy HIV-1 seropositive patients (Aboulker & Swart 1993), the hopes for preventing disease once infection is established are being dashed.

Much of the effort to combat AIDS has therefore focused on the development of a vaccine, an immunisation that can prevent the infection from occurring in the first place. However, although after only 10 years since its identification (Alizon et al. 1984; Barre-Sinoussi et al. 1983; Gallo et al. 1983, 1984; Hahn et al. 1984) more is known about the structure of HIV and the manner in which it interacts with the host than virtually any other virus, a vaccine remains elusive.

In this short review we will attempt to summarise the present state of anti-HIV vaccine development, with particular reference to the difficulties imposed by this most beguiling of viruses, and to describe the new approaches which promise the most success.

1. Early History of Anti-HIV Vaccine Development

Once the causative agent for AIDS had been identified as a retrovirus it seemed only a matter of time before an effective vaccine would be available to the general population. After all, previous work in murine and feline retrovirus systems had shown categorically that antibodies raised in response to the retroviral envelope glycoprotein could neutralise the virus *in vitro* (Fischinger et al. 1976; Hunsmann et al. 1974; Marciani et al. 1991) and indeed prevent infection or disease *in vivo* (Hunsmann et al. 1975a,b; Marciani et al. 1991).

The HIV-1 envelope glycoprotein gene *env* was very quickly identified, cloned, and the protein produced on a large scale using recombinant DNA techniques in a variety of expression systems (Barr et al. 1987; Barrett et al. 1989; Chakrabarti et al. 1986; Dewar et al. 1989; Morikawa et al. 1990; Putney et al. 1986). Antibodies to these proteins were indeed shown to neutralise the virus and even the precise epitopes to which neutralising antibod-

Table I. Approaches to HIV vaccine development

Immunogen type	Potential effector mechanism stimulated[a]	Efficacy against HIV/SIV in animal models
Whole inactivated virus	Neutralising antibody	No infection[b]
Native or recombinant viral subunits	Neutralising antibody[c]	No infection
Live recombinant virus vectors	Cytotoxic T lymphocytes[c]	No protection yet shown
Combination vaccine regimes	Cytotoxic T lymphocytes Neutralising antibody[c]	No infection
Synthetic peptide constructs	Neutralising antibody[c] Cytotoxic T lymphocytes	No disease
Live attenuated virus	'Natural' immune response[d]	No infection/disease
Naked DNA	Neutralising antibody[c] Cytotoxic T lymphocytes	Not done

a Immune responses able to eliminate virus or virus-infected cells.

b With few exceptions, effect probably mediated by cellular, not viral, components of the immunogen.

c Depending on which protein, peptide or gene is used.

d All immune parameters probably stimulated.

Abbreviation: SIV = simian immunodeficiency virus.

ies bind were described (Broliden et al. 1991; Ho et al. 1988; Javaherian et al. 1989). However, when used as a vaccine in the only available animal model for HIV-1 infection, the chimpanzee, the results were disappointing. Animals became infected after live challenge with even the homologous (immunising) HIV strain (Arthur et al. 1989; Berman et al. 1988). Achieving protective immunity was clearly not going to be a trivial task.

2. Animal Models

One of the major obstacles to developing and testing anti-HIV vaccines is, as mentioned above, the fact that the only real animal model for HIV-1 infection uses chimpanzees.

For practical, financial and ethical reasons, the number of chimpanzees available for research purposes is severely limited. Therefore, the vast majority of classical vaccine experiments (i.e. immunisation followed by challenge with live virus) have been performed in one of the simian immunodeficiency virus (SIV)/monkey systems (Daniel et al. 1990; Kurth et al. 1991). One isolate from commonly available rhesus macaques (SIVmac) has been studied in particular detail due to its property of inducing an AIDS-like disease in rhesus or cynomolgus monkeys (Chalifoux et al. 1987; Gardner 1989; Putkonen et al. 1992). Other retroviruses suitable for use in primate animal models include isolates from sooty mangabey monkeys (SIVsm), pig-tailed macaques (SIVmne), African green monkeys (SIVagm) and even HIV-2 (Hirsch et al. 1989; Kraus et al. 1989; Kuller et al. 1990; Putkonen et al. 1989).

Although there is no guarantee that a vaccine development shown to work in the SIV model will be directly applicable to the HIV/human system, most of the successful (and unsuccessful) vaccine experiments have been based on the SIV model. The use of PBL-SCIDhu mice (severely compromised immunodeficient mice carrying transplanted human lymphocytes) which are infectible with HIV-1 (Safrit et al. 1992), and the recently described (but not fully developed) HIV-1/pig-tailed macaque system (Agy et al. 1992) may allow vaccines based on HIV-1 itself to be evaluated on a large scale before testing in chimpanzees and humans.

3. Protective Mechanisms

One of the high priorities in anti-HIV vaccine research is to identify the correlates of immunity if they exist [i.e. the particular immune mecha-

nism(s) capable of protecting the host from infection or disease].

The known defence mechanisms which constitute the repertoire of the immune system have been studied in great detail in HIV-infected patients but the identity of those able to protect is still a matter of debate. As mentioned above, the early assumption that one needed only to stimulate the production of neutralising antibody to achieve protection unfortunately appears to be wrong. Certainly, experiments where high titre neutralising antibodies have been transferred to animals prior to challenge with HIV or SIV show that under these conditions antibodies can protect (Emini et al. 1992; Putkonen et al. 1991a). However, because of the high variability of HIV-1, particularly in those regions responsible for binding neutralising antibodies (e.g. the so-called V3 loop), it would be very difficult to artificially stimulate a comprehensive neutralising antibody response able to eliminate all strains with which one might come into contact.

The *in vivo* role of mechanisms such as antibody-dependent cellular cytotoxicity (ADCC) which can eliminate HIV-infected cells with broad specificity is still unknown (Ljunggren et al. 1989; Norley et al. 1990). Attention now appears to be focusing on the stimulation of HIV-specific cytotoxic T lymphocytes (CTL) by vaccines. CTLs, which are specific for short peptide fragments presented at the surface of the infected cell by the major histocompatibility complex (MHC)-I molecule, are able to lyse infected cells with great efficiency. This crucial immune response has the advantage that CTLs may recognise epitopes from virtually any viral protein, including regulatory proteins and the less variable structural proteins such as the core protein *gag* (Clerici et al. 1991; Culmann et al. 1991; Nixon et al. 1988; Walker et al. 1989). In terms of vaccine development, CTLs are unfortunately more difficult to stimulate due to the requirement for endogenous processing of the viral protein before association with the MHC-I molecule and presentation at the cell surface. This processing normally occurs only when a cell is actively infected with a replicating virus. However, as we shall discuss later, modern approaches to immunisation appear to be able to circumvent this requirement.

4. Vaccine Approaches

Table I lists the major types of vaccination currently being evaluated in HIV/SIV systems. Each has its advantages and disadvantages and it is a matter of some debate which is the most suitable or effective. It is worthwhile to describe each of these approaches in turn.

4.1 Whole Inactivated Virus

Whole inactivated virus is probably the simplest form of vaccine and historically one of the most successful.

As a result of the integrating nature of the retroviral genome, concerns about incomplete inactivation and the difficulties in growing HIV on a large scale, the use of whole inactivated HIV-1 as a vaccine was not initially seriously considered in this age of recombinant DNA technology. It is therefore somewhat ironic that the first demonstration of complete protection from infection was achieved in the SIVmac system using an inactivated virus vaccine (Murphey-Corb et al. 1989). Many laboratories repeated and extended these findings (Desrosiers et al. 1989; Hartung et al. 1992; Johnson et al. 1992; Stott et al. 1990), causing an enormous boost in optimism that a vaccine was at last achievable. However, the discovery that protection in these experiments was in the large part due to human cell proteins incorporated into both the immunising virus preparation and the challenge virus dashed these hopes (Stott 1991).

To date, nobody has successfully protected monkeys against challenge with cell-free SIVmac grown in monkey cells using an inactivated virus preparation (Le Grand et al. 1992; Norley, unpublished observation). It may transpire, however, that the SIVmac model is in some way not representative of the other primate viruses, since protection has been achieved against HIV-2 grown in monkey cells using an inactivated virus vaccine (Putkonen et al. 1991b). Inactivated virus may ad-

Table II. Known HIV and simian immunodeficiency virus (SIV) neutralising (Neut) and antibody-dependent cellular cytotoxic (ADCC) antibody epitopes

Amino acid positions (isolate)[a]	Amino acid sequence	Activity[b]	Reference
SIVmac gp130			
171-180 (251)	KFTMTGLKRD	Neut	Benichou et al. (1992)
176-188 (251)	GLKRDKTKEYNET	Neut	Benichou et al. (1992)
170-190 (32H)	NMTGLKRDKTKEYNETWYSTD	Neut	Kent et al. (1992)
HIV-1 gp120			
169-183 (HXB2R)	VQKEYAFFYKLDIIP	Neut	Fung et al. (1992)
247-267 (HXB2R)	CTHGIRPVVSTQLLLNGSLAE	Neut, ADCC	Ho et al. (1988)
HIV-1 gp120 (V3 loop)			
295-327 (LAI)	QSVEINCTRPNNNTRKSIRIQRGPGRAFVTIGK	Neut	Wang et al. (1991)
296-312 (LAI)	SVEINCTRPNNNTRKSI	Neut	Ho et al. (1987)
301-319 (LAI)	CTRPNNNTRKSIRIQRGPG	Neut	Hart et al. (1990)
301-319 (LAI)	CTRPNNNTRKSIRIQRGPG(Y)	Neut	Palker et al. (1988)
301-319 (LAI)	CTRPNNNTRKSIRIQRGPG(Y)	Neut	Palker et al. (1989)
306-329 (LAI)	NNTRKSIRIQRGPGRAFVTIGKIG(C)	Neut	Rusche et al. (1988)
306-329 (LAI)	NNTRKSIRIQRGPGRAFVTIGKIG	Neut	Matsushita et al. (1988)
306-329 (LAI)	NNTRKSIRIQRGPGRAFVTIGKIG(C)	Neut	Javaherian et al. (1989)
306-329 (LAI)	NNTRKSIRIQRGPGRAFVTIGKIG(C)	Neut	Javaherian et al. (1990)
306-329 (LAI)	NNTRKSIRIQRGPGRAFVTIGKIG	Neut	Nardelli et al. (1992)
306-318 (LAI)	NNTRKSIRIQRGP	Neut	Skinner et al. (1988)
306-329 (LAI)	NNTRKSIRIQRGPGRAFVTIGKIG	Neut	Defoort et al. (1992)
306-329 (LAI-RF-LAI)	NNTRKSIRIQRGPGRVIYATGKIG(C)	Neut	Javaherian et al. (1989)
308-328 (LAI)	TRKSIRIQRGPGRAFVTIGKI	Neut	Linsley et al. (1988)
309-323 (LAI)	RKSIRIQRGPGRAFV	Neut	Broliden et al. (1990)
309-323, 315-320 (LAI)	RKSIRIQRGPGRAFV	Neut	Akerblom et al. (1990)
309-323 (LAI)	RKSIRIQRGPGRAFV	Neut	Broliden et al. (1992)
309-323 (LAI-RF)	KSIRIQRGPGRVIY(C)	Neut	Javaherian et al. (1989)
312-326 (LAI)	IRIQRGPGRAFVTIG	Neut	Goudsmit et al. (1988)
307-322 (HXB2R)	IRIQRGPGRAFVTIGK	Neut	Ohno et al. (1991)
308-322 (HXB2R)	RIQRGPGRAFVTIGK	Neut, ADCC	Liou et al. (1989)
308-322 (HXB2R)	RIQRGPGRAFVTIGK	Neut	Kenealy et al. (1989)
308-322 (HXB2R)	RIQRGPGRAFVTIGK	Neut	Durda et al. (1990)
312-317	GPGRAF	Neut	Javaherian et al. (1990)
312-317	GPGRAF	Neut	White-Scharf et al. (1993)
295-325 (MN)	ESVQINCTRPNYNKRKRIHI GPGRAFYTTKN	Neut	Wang et al. (1991)
299-338 (MN)	INCTRPNYNKRKRIHI GPGRAFYTTKNIIGTIRQAHCNIS	Neut	White-Scharf et al. (1993)
301-319 (MN)	CTRPNYNKRKRIHI GPGRA	Neut	Palker et al. (1989)
304-327 (MN)	PNYNKRKRIHI GPGRAFYTTKNII	Neut	Nardelli et al. (1992)
304-326 (SF2)	NNTRKSIYI GPGRAFHTTGRIIG(C)	Neut	White-Scharf et al. (1993)

Table II. Contd

Amino acid positions (isolate)[a]	Amino acid sequence	Activity[b]	Reference
306-328 (MN)	YNKRKRIHI GPGRAFYTTKNIIG(C)	Neut	White-Scharf et al. (1993)
306-328 (MN)	YNKRKRIHI GPGRAFYTTKNIIG(C)	Neut	Javaherian et al. (1989)
306-328 (MN)	YNKRKRIHI GPGRAFYTTKNIIG(C)	Neut, ADCC	Scott et al. (1990)
306-328 (MN)	YNKRKRIHI GPGRAFYTTKNIIG(C)	Neut	Javaherian et al. (1990)
306-328 (MN)	YNKRKRIHI GPGRAFYTTKNIIG(C)	Neut	Profy et al. (1990)
309-325 (MN)	RKRIHI GPGRAFYTTKN	Neut	Broliden et al. (1992)
310-314 (MN)	KRIHI	Neut	Gorny et al. (1991)
311-325 (MN)	RIHI GPGRAFYTTKN	Neut	Ohno et al. (1991)
311-315 (MN)	RIHI G	Neut	White-Scharf et al. (1993)
312-318 (MN)	IXI GPGR	Neut	White-Scharf et al. (1993)
312-322 (MN)	IHI GPGRAFYT(C)	Neut	Profy et al. (1990)
313-320 (MN)	HI GPGRAF	Neut	White-Scharf et al. (1993)
313-318 (MN)	HI GPGR	Neut	Gorny et al. (1991)
321-335 (MN)	YTT(C)NIIGTIRQAHC	Neut	Broliden et al. (1992)
314-336 (RF)	NNTRKSI TKGPGRVIYATGQIIG(C)	Neut	Javaherian et al. (1989)
314-336 (RF)	NNTRKSI TKGPGRVIYATGQIIG	Neut	Nardelli et al. (1992)
317-329 (RF)	(C)RKSI TKGPGRVIY	Neut	Palker et al. (1989)
320-331 (RF)	I TKGPGRVI(YAT)	Neut	Goudsmit et al. (1988)
320-329 (RF)	I TKGPGRVIY(C)	Neut	Javaherian et al. (1989)
423-437 (HXB2R)	IINMWQKVGKAMYAP	Neut	Sun et al. (1989)
451-477 (HXB2R)	GLLLTRDGGNSNNESEIFRPGGGDMRD	Neut	Ho et al. (1987)
487-508 (LAI)	ELYKYKVVKIEPLGVAPTKAKR	Neut	Charbit et al. (1990)
489-508 (HXB2R)	VKIEPLGVAPTKAKRRVVQR	Neut	Broliden et al. (1992)
496-525 (HXB2R)	VAPTKAKRRVVQREKRAVGIGALFLGFLGA	Neut	Chanh et al. (1986)
496-525 (HXB2R)	VAPTKAKRRVVQREKRAVGIGALFLGFLGA	Neut	Ho et al. (1987)
HIV-1 gp41			
566-590 (LAI)	AQQHLLQLTVWGIKQLQARILAVER	ADCC	Wood et al. (1990)
579-604 (HXB2R)	RILAVERYLKDQQLLGIWGCSGKLIC	ADCC	Tyler et al. (1990)
598-609 (Z3)	LGLWGCSGKLIC	Neut	Schrier et al. (1988)
609-625 (HXB2R)	PWNASWSNKSLEQIWNH	Neut	Ho et al. (1987)
644-663 (HXB2R)	(C)SLIEESQNQQEKNEQELLEL	ADCC	Tyler et al. (1990)
652-666 (HXB2R)	EESQNQQEKNEQELLELDKWASLWN	Neut	Broliden et al. (1992)
721-744 (HXB2R)	LPTPRGPDRPEGIEEEGGERDRDR	Neut	Ho et al. (1987)
721-745 (HXB2R)	LPTPRGPDRPEGIEEEGGERDRDRS	ADCC	Evans et al. (1989b)
728-745 (HXB2R)	(Y)DRPEGIEEEGGERDRDRS	Neut	Chanh et al. (1986)
728-745 (HXB2R)	DRPEGIEEEGGERDRDRS	Neut	Ho et al. (1987)
728-745 (HXB2R)	DRPEGIEEEGGERDRDRS	Neut	Evans et al. (1989a)
728-745 (HXB2R)	DRPEGIEEEGGERDRDRS	Neut	Evans et al. (1989a)
728-745 (HXB2R)	DRPEGIEEEGGERDRDRS	Neut	Evans et al. (1989a)

Continued on next page

Table II. Contd

Amino acid positions (isolate)[a]	Amino acid sequence	Activity[b]	Reference
728-745 (HXB2R)	DRPEGIEEEGGERDRDRS	Neut	Dalgleish et al. (1988)
732-746 (HXB2R)	GIEEEGGERDRDRSI	Neut	Broliden et al. (1992)
824-848 (LAI)	AIAVAEGTDRVIEVVQGACRAIRHI	Neut	Boyer et al. (1989)
HIV-1 p17 (*gag*)			
12-19 (HXB2R)	ELDRWEKI	Neut, ADCC	Papsidero et al. (1989)
17-22 (HXB2R)	EKIRLR	Neut, ADCC	Papsidero et al. (1989)
92-109 (LAV)	IEIKDTKEALDKIEEEQN	Neut	Sarin et al. (1986)
100-105 (HXB2R)	ALDKIE	Neut, ADCC	Papsidero et al. (1989)

a Amino acid positions according to the published sequences of the isolates used (when known), as shown in brackets.
b Antiviral activity of the specific antibodies.

ditionally have a role as an immunotherapeutic agent, hopefully stimulating the immune system of infected people to the point that the infection remains under control (Salk 1987).

4.2 Subunit Vaccines

As described earlier, although the use of recombinant DNA technology makes possible the production of virtually any viral protein on a large scale, the initial trials using HIV-1 subunit vaccines were disappointing. These failures may have been partially the result of the expression systems used to produce the recombinant proteins.

The HIV-1 envelope glycoprotein is one of the most heavily glycosylated proteins known, and it is becoming increasingly clear that the pattern of glycosylation strongly influences the conformation of the protein and how antibodies bind (Davis et al. 1990). The use of viral vectors able to induce production of HIV envelope proteins in eukaryotic cells (e.g. baculovirus recombinants in insect cells or vaccinia virus recombinants in mammalian cells) allows glycosylation to occur. Subunit vaccines produced by such systems have indeed been shown to protect chimpanzees from infection (Berman et al. 1990) and are currently undergoing phase I and II clinical trials for safety and immunogenicity or as immunotherapeutic agents. However, it is important to keep in mind that an immunogen which protects chimpanzees against a low dose of cell-free homologous virus after an optimal and lengthy series of immunisations will not necessarily constitute an effective vaccine in the field.

4.3 Live Recombinant Vaccines

If, as seems likely, the stimulation of an antibody response alone by recombinant proteins does not give a sufficient degree of protection, strategies to stimulate the cellular arm of the immune system will probably be needed.

As we know, CTLs are normally stimulated only by active infection, and one way to achieve a CTL response to HIV is therefore to infect with a relatively harmless virus expressing HIV genes. For example, animals or humans infected by a vaccinia virus which has been genetically manipulated to carry the HIV-1 *env* gene will respond with a CTL response to HIV-1 as well as to vaccinia itself (Gotch et al. 1991; Zarling et al. 1987). For a number of practical reasons the vaccinia vector is perhaps not the ideal vehicle for a general use live recombinant virus, but other vectors, such as adenoviruses, show promise (Natuk et al. 1992; Prevec et al. 1991). In contrast to the situation with subunit vaccines, however, live recombinant vaccines appear to be poor inducers of anti-HIV antibody responses (Graham et al. 1992).

4.4 Combination Vaccines

As well as inducing CTLs, live recombinant vaccines are good stimulators of immunological memory, and one obvious vaccination regime is therefore to first infect with a recombinant virus and then boost the response with the corresponding subunit vaccine.

Such approaches have been used to achieve protection against infection with SIVmne in monkeys (Hu et al. 1992) and HIV-1 in chimpanzees (Girard et al. 1991). Indeed, human trials are currently underway using a variation of the live recombinant with subunit boost approach.

4.5 Synthetic Peptides

Given the sequence of a virus genome it is possible to chemically synthesise regions of the corresponding proteins and to identify those regions responsible for inducing the different immune mechanisms. Such 'epitope mapping' has been performed for many of the known HIV and SIV isolates, and a large number of neutralising, ADCC, T helper and CTL epitopes are known. Table II is a comprehensive (although possibly incomplete) listing of the known HIV and SIV epitopes known to be recognised by functional antibodies (neutralising or ADCC activating). Table III similarly lists those epitopes which activate and are recognised by CTLs

Using this information one can pick and choose the regions to be included in a vaccine, omitting for example regions of the envelope glycoprotein responsible for eliciting infection-enhancing antibodies (Robinson et al. 1991; Takeda et al. 1992). In terms of antibody induction, peptides are of course no better (or even worse) than recombinant proteins with regard to their breadth of immunogenicity, being in particular restricted to linear, continuous epitopes. Recent technologies, such as the use of multiple antigenic peptides, allow the immunogenicity of synthetic peptides to be enhanced (Wang et al. 1991), but real promise for peptides in terms of vaccines lies in their ability to circumvent the need for active infection to stimulate CTLs.

If chosen correctly, synthetic peptides mimic exactly the processed protein fragments presented by the MHC-I molecule to a CTL. There are reports of CTL induction by injection of peptide alone (Sastry et al. 1992), although the use of palmitic acid–derived peptides or liposomes appears to be an effective method of diverting peptides into the endogenous processing pathway to stimulate CTLs (Deres et al. 1989; Schild et al. 1991).

Multiepitopic peptides consisting of neutralising, CTL and T helper epitopes in tandem, or of conserved envelope epitopes, have also been tested in animal models with some success (Hart et al. 1990; Shafferman et al. 1991, 1992).

In addition, it has recently been demonstrated that hybrid Ty virus–like particles derived from yeast cells carrying the gene fragment coding for the HIV-1 principal neutralising domain (V3 loop) stimulate a strong CTL response when injected into mice (Layton et al. 1993). Whether analogous constructs can protect against infection in one of the animal models for AIDS should soon be known.

Finally, an integral part of the ongoing combination immunisation trials mentioned above is the use of peptides corresponding to the V3 loop of HIV-1 for the subunit boost.

4.6 Attenuated Virus

In principle, the immune response most able to prevent infection is the response stimulated by the replicating virus itself. Infection with an attenuated or apathogenic variant of a virus is an extremely powerful method of vaccination, as demonstrated by the global elimination of smallpox and the efficacy of the Sabin polio vaccine. The problem with HIV is not that attenuated strains do not exist, since these can be produced by genetic manipulation, rather it is that the HIV genome integrates in human chromosomes and, although not yet observed, can potentially cause lymphoma, as with human T cell leukaemia virus type I.

Initial and understandable resistance to the idea of using a live attenuated HIV vaccine is now beginning to wane with the demonstration in the SIVmac model that prior infection with a SIVmac

Table III. Known HIV and simian immunodeficiency virus (SIV) cytotoxic T lymphocyte (CTL) epitopes

Amino acid positions (isolate)[a]	Amino acid sequence	Species	Reference
SIV *gag*			
171-195 (251)	VPGFQALSEGCTPYDINQMLNCVGD	Rhesus	Yamamoto et al. (1990); Shen et al. (1991); Miller et al. (1991); Yamamoto et al. (1992)
179-190 (251)	EGCTPYDINQML	Rhesus	Yamamoto et al. (19920
182-190 (251)	TPYDINQML	Rhesus	Miller et al. (1991)
264-278 (SIVmm142)	RRWIQLGLQK(S)VRMY	Human	Nixon et al. (1990)
SIV *nef*			
108-123 (251)	LRAMTYKLAIDMSHFI	Rhesus	Bourgault et al. (1992)
155-169 (251)	DWQDYTSGPGIRYPK	Rhesus	Bourgault et al. (1992)
164-178 (251)	GIRYPKTFGWLWKLV	Rhesus	Bourgault et al. (1992)
HIV-2 *gag*			
265-279 (ROD)	RRWIQIGLQK(S)VRMY	Human	Nixon et al. (1990)
HIV-1 *gag*			
18-42 (BH10)	KIRLRPGGKKKYKLKHIVWASRELE	Human	Johnson et al. (1991b)
69-93 (BH10)	QTGSEELRSLYNTVATLYCVHQRIE	Human	Johnson et al. (1991b)
86-115 (LAI)	Y(S)VHQRI(DV)KDTKEAL(E)KIEEEQNKSKKKA	Human	Achour et al. (1990)
140-152 (BH10)	GQMVHQAISPRTL	Human	Littaua et al. (1991)
143-164 (BH10)	VHQAISPRTLNAWVKVVEEKAF	Human	Johnson et al. (1991b)
153-174 (BH10)	NAWVKVVEEKAFSPEVIPMFSA	Human	Johnson et al. (1991b)
173-194 (BH10)	SALSEGATPQDLNTMLNTVGGH	Human	Johnson et al. (1991b)
193-214 (BH10)	GHQAAMQMLKETINEEAAEWDR	Human	Johnson et al. (1991b)
193-203 (LAI)	GHQAAMQMLKE	Human	Claverie et al. (1988)
219-233 (LAI)	HAGPIAPGQMREPRG	Human	Claverie et al. (1988)
253-274 (BH10)	NPPIPVGEIYKRWIILGLNKIV	Human	Johnson et al. (1991b)
263-284 (BH10)	KRWIILGLNKIVRMYSPTSILD	Human	Johnson et al. (1991b)
263-277 (ELI)	KRWIIVGLNKIVRMY	Human	Nixon et al. (1990)
265-279 (SF2)	KRWIILGLNKIVRMY(C)	Human	Nixon et al. (1988)
265-279 (SF2)	KRWIILGLNKIVRMY	Human***	Nixon et al. (1990)
305-314 (BH10)	RAEQASQEVK	Human	Johnson et al. (1991b)
313-334 (BH10)	VKNWMTETLLVQNANPDCKTIL	Human	Johnson et al. (1991b)
418-433 (LAI)	KEGHQMKDCTERQANF	Human	Claverie et al. (1988)
446-460 (LAI)	GNFLQSRPEPTAPPF	Human	Claverie et al. (1988)
HIV-1 *pol*			
172-196 (LAI)	IETVPVKLKPGMDGPKVKQWPLTEE	Human	Walker et al. (1989)
203-219 (LAI)	EICTEMEKEGKISKIGP	Mouse	Hosmalin et al. (1992)
203-219 (LAI)	EICTEMEKEGKISKIGP	Mouse	Hosmalin et al. (1992)
203-219 (LAI)	EICTEMEKEGKISKIGP	Mouse	Hosmalin et al. (1990)
203-219 (LAI)	EICTEMEKEGKISKIGP	Human	Hosmalin et al. (1990)
342-349 (LAI)	NPDIVIYQ	Human	Walker et al. (1989)

Table III. Known HIV and simian immunodeficiency virus (SIV) cytotoxic T lymphocyte (CTL) epitopes

Amino acid positions (isolate)[a]	Amino acid sequence	Species	Reference
359-383 (LAI)	DLEIGQHRTKIEELRQHLLRWGLTT	Human	Walker et al. (1989)
461-485 (LAI)	PLTEEAELELAENREILKEPVHGVY	Human	Walker et al. (1989)
495-519 (LAI)	EIQKQGQGQWTYQIYQEPFKNLKTG	Human	Walker et al. (1989)
681-695 (LAI)	ESELVNQIIEQLIKK	Mouse	Hosmalin et al. (1992)
HIV-1 *env*			
112-124 (BH10)	HEDIISLWDQSLK	Human	Clerici et al. (1991)
301-324 (MN)	CTRPNYNKRKRIHIGPGRAFYTTK	Mouse	Hart et al. (1991)
301-325 (LAI)	CTRPNNNTRKSIRIQRGPGRAFVTI	Mouse	Hart et al. (1991)
306-329 (LAI)	NNTRKSIRIQRGPGRAFVTIGKIG	Mouse	Defoort et al. (1992)
308-322 (HXB2R)	RIQRGPGRAFVTIGK	Mouse	Takahashi et al. (1988)
308-322 (HXB2R)	RIQRGPGRAFVTIGK	Mouse	Takahashi et al. (1990)
308-322 (HXB2R)	RIQRGPGRAFVTIGK	Human	Clerici et al. (1991)
381-392		Human	Walker & Plata (1990)
381-392		Human	Takahashi et al. (1991b)
428-443 (BH10)	KQIINMWQEVGKAMYA	Human	Clerici et al. (1991)
587-599 (LAI)	AVERYLKDQQLLG	Human	Johnson et al. (1991a)
765-778 (NL43)	SYHRLRDLLLIVTR	Human	Takahashi et al. (1991a)
776-789 (RF)	SYHRLRDLLLIVVR	Human	Takahashi et al. (1991a)
768-778 (NL43)	RLRDLLLIVTR	Human	Takahashi et al. (1991b)
779-789 (RF)	RLRDLLLIVVR	Human	Takahashi et al. (1991b)
782-792 (CDC4)	RLRDLLLIVAR	Human	Takahashi et al. (1991b)
834-848 (BH10)	DRVIEVVQGAYRAIR	Human	Clerici et al. (1991)
HIV-1 *nef*			
73-82 (NL432)	QVPLRPMTYK	Human	Koenig et al. (1990)
73-82(LAI)	QVPLRPMTYK	Human	Culmann et al. (1991)
79-94(83-94)[LAI]	MTYKAAVDLSHFLKEK	Human	Culmann et al. (1991)
113-128 (LAI)	WIYHTQGYFPDWQNYT	Human	Culmann et al. (1989)
113-128 (Z321)	WVYHTQGFFPDWHNYT	Human	Culmann et al. (1991)
113-128 (Z6)	WVYNTQGIFPDWQNYT	Human	Culmann et al. (1991)
115-125 (LAI)	YHTQGYFPDWQ	Human	Culmann et al. (1991)
117-128 (LAI)	TQGYFPDWQNYT	Human	Culmann et al. (1991)
126-138 (LAI)	NYTPGPGVRYPLT	Human	Culmann et al. (1991)
132-147 (LAI)	GVRYPLTFGWCYKLVP	Human	Culmann et al. (1991)

a Amino acid positions according to the published sequences of the isolates used (when known), as shown in brackets.

mutant devoid of the *nef* gene, as well as being apparently safe over a period of years, prevents subsequent infection with even a high dose of the pathogenic virus (Desrosiers 1992). This is by far the most impressive protection demonstrated to date with a vaccine in the HIV/SIV field. With other candidate genes available for excision thus rendering the virus even safer and perhaps incapable of integration (Vogel et al. 1993), the use of a live recombinant HIV-1 vaccine, in the absence of any other effective measure, deserves serious consideration.

4.7 Naked DNA

The problems associated with anti-HIV vaccine development using traditional means, which at times appear almost insurmountable, suggests that entirely new approaches to vaccination may be needed.

One such approach recently developed appears to circumvent the restrictions and dangers associated with the use of live recombinant or attenuated viruses to stimulate cellular immunity. Surprisingly, it has been shown that if naked viral DNA is injected into an animal it is somehow taken up by the cells, transcribed and translated to produce proteins by normal mechanisms which are then presumably processed and presented via the endogenous pathway to stimulate a CTL response. Using such a system, mice injected with DNA coding for influenza nucleoprotein were shown to be immune to lethal challenge with a heterologous virus strain (Ulmer et al. 1993). This system is presently being evaluated in the SIV animal model, with refinements such as the use of high pressure aerosol delivery equipment and gold particle bombardment.

One interesting modification of the use of naked DNA, not yet tested in animal models for AIDS, is the use of liposomes to deliver messenger RNA (mRNA) to the cell cytoplasm. The mRNA is then translated into protein which is processed and expressed and hence stimulates a CTL response (Martinon et al. 1993).

5. Conclusions

It is, to say the least, disappointing that 10 years after the identification of HIV as the causative agent for AIDS the epidemic continues unabated. Our previous successes in combating infectious agents, particularly in the field of antiviral vaccines, had perhaps lulled us into a false sense of security. It seemed that once the impressive arsenal of techniques available to the scientific community were brought to bear on HIV, a vaccine would not be long coming.

However, the last 10 years have, more than anything else, shown us how easily an entity as relatively simple as HIV can overcome the best that human ingenuity can offer. The breakthrough that is needed to quickly produce an effective, practical and inexpensive vaccine has not yet occurred. However, our basic knowledge of HIV and the manner in which it interacts with the host is accumulating rapidly. The limited number of successful vaccine studies performed under laboratory conditions in the different animal models demonstrate that it is indeed possible to elicit a state of sterilising immunity against HIV or its simian equivalents.

At the moment it is difficult to predict which, if any, of the current vaccination procedures being tested in laboratories will eventually become the basis for an effective and practical anti-HIV vaccine. It seems likely that the stimulation of both cellular and humoral immune responses will be necessary. In this case, strategies such as combination vaccines, modified synthetic peptides or naked DNA may be successful. However, if HIV strains attenuated by deletion of pathogenic genes can be shown to be absolutely safe, we may yet see by default the general use of a live HIV-1 vaccine in high risk populations.

Hopefully, it is only a matter of time before such approaches to vaccine development yield a product able to protect those millions at risk now and in the future from HIV infection and AIDS.

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